THIS NUMBER COMPLETES VOLUME XLVIII

JUN 22 1931

Medical Library

# BULLETIN

## OF THE

# JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XLVIII, No. 6 JUNE, 1931

## *Contents*

Programs of The Johns Hopkins Medical Society, 1930–1931 ..... 353

Treatment of Hemicrania (Migraine) by Removal of the Inferior Cervical and First Thoracic Sympathetic Ganglion. WALTER E. DANDY ..... 357

The Effect of the Injection of Urine from Pregnant Mammals on Ovulation in the Rabbit. F. F. SNYDER AND G. B. WISLOCKI ..... 362

A Human Tubal Egg, Unfertilized. WARREN H. LEWIS ..... 368

The Mechanism of the Ketogenic Diet in Epilepsy. EDWARD M. BRIDGE AND L. V. IOB ..... 373

Biological Studies of the Acid-fast Organism. S. A. PETROFF AND WM. STEENKEN, JR. ..... 390

Index ..... 395

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS

BALTIMORE, MARYLAND

*Made in United States of America*

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

# BULLETIN
## OF
# THE JOHNS HOPKINS HOSPITAL



MANAGING EDITOR, E. Cowles Andrus

EDITORIAL BOARD

Edward P. Carter
Charles R. Austrian
Arnold R. Rich
Paul G. Shipley
Frank R. Smith
George B. Wislocki



*All correspondence relating to the publication of papers* should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore, Md.

*Books for reviews* should be sent to The Editor at the above address.

*Twenty-five reprints*, with covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

*Correspondence concerning business matters* should be sent to The Johns Hopkins Press, Baltimore, Maryland, U. S. A.

*This periodical is issued monthly.* Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

*Subscription price.* In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

*New Subscriptions and renewals* are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefore adjusted accordingly.

*Subscriptions should be renewed promptly*—To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Agents for the British Empire, except Canada: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, W.C. 2, London.

## THE JOHNS HOPKINS PRESS
BALTIMORE, MARYLAND, U. S. A.

# PROGRAMS AND LECTURES, 1930–1931

## PROGRAMS OF THE JOHNS HOPKINS MEDICAL SOCIETY

*October 13, 1930*

1. A Graphic Demonstration of Cerebellar Ataxia. Gordon Holmes, M.D., London, England.
2. Di-hydranol. Control of Intestinal Putrefaction in Man by Oral Administration of Di-hydroxyphenyl N-heptane. Veader Leonard, M.D., and William A. Feirer, M.D.
3. The Glomerular Development of the Vertebrate Kidney in Relation to Habitat. E. K. Marshall, Jr., M.D.

*November 10, 1930*

1. Thoracoplasty in the Treatment of Tuberculosis. Robert Miller, Jr., M.D.
2. The Clinical Significance of Latent Tuberculosis. Eugene L. Opie, M.D., Philadelphia, Pa.

*December 8, 1930*

1. The Oral, Intragastric and Intraduodenal Administration of Histamine in Human Subjects. Moses Paulson, M.D.
2. The Biology of the Tubercle Bacillus. S. A. Petroff, M.D., Trudeau, New York.

## PROGRAM OF THE JOINT MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

AND

## THE HISTORICAL CLUB OF THE JOHNS HOPKINS HOSPITAL

*January 12, 1931*

1. Introductory Remarks. William H. Welch, M.D.
2. The Cult of Asklepios. Stephen d'Irsay, M.D.

## PROGRAM OF THE JOHNS HOPKINS MEDICAL SOCIETY

*February 9, 1931*

1. Improved Method in the Laboratory Diagnosis of Syphilis. Harry S. Eagle, M.D.
2. A Consideration of Coronary Thrombosis and its Sequelae and of Other Causes of Pain in the Cardiac Area. E. Libman, M.D., New York City, N. Y.

*March 9, 1931*

1. The Surgical Treatment of Snake Bites. Dudley Jackson, M.D., San Antonio, Texas.
2. Studies on the Cellular Reactions in Tuberculosis. Florence Sabin, M.D., New York City, N. Y.

*April 13, 1931*

1. Clinical Treatment of Syphilis. John Reisinger, M.D.
2. An Experimental Study of Hereditary Dwarfism in Mice. Philip E. Smith, M.D., Columbia University, and E. C. MacDowell, Sc.D., Cold Spring Harbor, N. Y.

*May 11, 1931*

1. Election of Officers.
2. Meningeal Permeability with Special Reference to Bromide and Calcium. S. Katzenelbogen, M.D.
3. The Relation of Streptococci to Arthritis and Rheumatic Fever. Russell L. Cecil, M.D., Cornell University Medical School.

## LECTURES UNDER THE AUSPICES OF THE HERTER LECTURE FOUNDATION

*October 16, 1930*

Dermatomes in Man. O. Foerster, M.D., Breslau, Germany.

*December 8, 1930*

The Significance of Sinanthropus. G. Elliott Smith, M.D., London, England.

## THE CHARLES E. DOHME MEMORIAL LECTURESHIP

*April 16, 1931*

Insulin. Professor Karl Freudenberg, Heidelberg, Germany.

*April 17, 1931*

Optical Activity and Configuration of Oxy- and Amino-acids. Professor Karl Freudenberg, Heidelberg, Germany.

*April 18, 1931*

Recent Researches on Cellulose and Starch. Professor Karl Freudenberg, Heidelberg, Germany.

## THE WILLIAM SYDNEY THAYER AND SUSAN READ THAYER LECTURESHIP IN CLINICAL MEDICINE

*May 11, 1931*

The Natural History of Glomerular Nephritis. Thomas Addis, M.D., San Francisco, Cal.

*May 12, 1931*

Questions in Connection with the Prognosis, Treatment and Etiology of Glomerular Nephritis. Thomas Addis, M.D., San Francisco, Cal.

## THE JOHNS HOPKINS MEDICAL HISTORICAL SOCIETY

*December 18, 1930*

Gleanings from Medical and Scientific Incunabula. Arnold C. Klebs, M.D., Nyon, Switzerland.

*March 23, 1931*

1. Medical Material from Matheo Aleman's "The Rogue," translated by James Mabbe. John Rathbone Oliver, M.D.
2. Human Credulity as Illustrated by Belief in Witchcraft. Theodore Diller, M.D., Pittsburgh, Pa.

*May 14, 1931*

1. The Use of the Hebrew Language in Medical Literature. Harry Friedenwald, M.D.
2. Development of Medical Field Service, Especially in Connection with the Evacuation of Wounded of a Field Army. Col. Charles R. Reynolds, M.C., Carlisle Barracks, Pa.

## LECTURES BY SIR D'ARCY POWER, K.B.E., F.R.C.S. ENG. VISITING LECTURER IN THE INSTITUTE OF THE HISTORY OF MEDICINE

*November 19, 1930*

The Essentials of Medical Biography.

*November 25, 1930*

Medical Bibliography.

*December 2, 1930*

The Meals of Our Ancesters.

*December 3, 1930*

"Aristotle's Masterpiece."

*December 10, 1930*

Medical Iconography.

*December 17, 1930*

The Growth of a Hospital.

## PROGRAMS OF THE JOHNS HOPKINS MEDICAL RESEARCH SOCIETY

*October 24, 1930*

1. The Transmission of the Common Cold by Means of a Filterable Virus. Perrin H. Long, M.D.
2. The Urobilin Excretion in Pneumonia. Hugh Josephs, M.D.

*November 21, 1930*

1. The Relation of the Spleen to Methaemoglobin Formation. G. A. Harrop, M.D.
2. The Persistence of Acquired Immunity After the Abolition of Allergy by Desensitization. A. R. Rich, M.D., and F. B. Jennings, Jr., M.D.
3. The Demonstration of a New Stain for Cell Surfaces. Jonas Friedenwald, M.D.

*January 30, 1931*

1. Experimental Studies on Sleep. Curt Richter, M.D.
2. Studies on the Adrenal Cortical Hormone. G. A. Harrop, M.D.
3. The Pharmacological Action of Pituitary Principles. E. M. K. Geiling, M.D.

*March 6, 1931*

1. The Circulation of the Aqueous Tumor. Jonas Friedenwald, M.D.
2. The Origin of the Fat Present in Milk. L. E. Holt, M.D.

*April 10, 1931*

A Study of the Effects of Transfusion with Plasma. Hugh Josephs, M.D.

*May 15, 1931*

1. The Correlation of Structure and Function in Hearing. Stacy Guild, M.D.
2. The Significance of Encapsulated Forms of Haemolytic Streptococci. F. B. Jennings, Jr., M.D.
3. The Mechanism which Prevents the Spread of Bacteria in the Immune Body. A. R. Rich, M.D.

# TREATMENT OF HEMICRANIA (MIGRAINE) BY REMOVAL OF THE INFERIOR CERVICAL AND FIRST THORACIC SYMPATHETIC GANGLION[1]

WALTER E. DANDY

*From the Neuro-Surgical Department of the Johns Hopkins University and Hospital*

The reasoning behind the operative treatment here suggested for migraine was as follows: The actual pain, so perfectly restricted to one side of the head (unless both sides are involved), must indicate an affection of nerves which carry sensation. No sensory cranial or spinal nerves could be the source of this widespread pain because of the extensive domain involved. A trigeminal pain is eliminated for this reason, as well as by the fact that evidences of pain in the known sensory distribution of this nerve are invariably absent. When air is injected intraspinally, a severe sharp hemicranial pain is produced when the air reaches the cerebral subarachnoid space. It later becomes generalized when the air reaches both sides of the cerebral subarachnoid space. Even the passage of the air from place to place in the subarachnoid space can be defined by the pain produced. This must mean that pain-carrying nerves are in the meninges. Moreover, there is no such pain when air is introduced into the lateral ventricles; hence there are no nerves there. The only possible nerve supply to the meninges is through the sympathetic system.

The fact that in many cases of migraine there is the extensive cerebral involvement, as evidenced by hemianopsia (sometimes becoming bilateral), aphasia, etc., suggested a possible vascular spasm of certain cerebral arteries. Were this true, a nerve supply to the arteries would be required. It was difficult for us to postulate any other anatomical background for such widespread attacks. As a matter of fact there have been many theories to explain this widespread involvement of the brain, in conjunction with the hemicrania, but the proof by actual experiment has been lacking for all, and without such proof any theory,

[1] Submitted for publication May 1, 1931.

including the one just presented, is little more than valueless. However, since the reasoning from the various angles seemed to concentrate to the same point—the sympathetic system—the removal of the cerebral sympathetic nerves seemed warranted. Moreover, in the two cases here reported the patients showed during the attacks certain objective stigmata which unquestionably indicated involvement of the sympathetic nervous system.

*Case I.* A large, well developed, healthy, somewhat nervous man, aged 51, was referred by Professor Longcope. Since childhood he had had severe attacks of pain exclusively on the right side of the head. Since the age of 17 or 18, these attacks have gradually increased in frequency and severity. Those at the present time are of essentially the same character, though of so much greater severity that he feels they are no longer bearable and urgently seeks relief. The pains are in the right frontal, orbital and maxillary regions. At first they were like a mild neuralgia and were induced by thought, by exposure to cold, or by hard study and mental concentration. The attacks would then last for about twenty minutes, after which there would be complete relief for several months. At times there might be periods of nightly recurrence of the pain over a period of a week or ten days. Now they come nearly every night over periods of one to four months, and frequently also in the day time during this interval. Each attack now lasts for an hour or more. From November, 1929, to March, 1930, there was not a day in which one or more attacks did not develop. From November 15th to December 15th of this period the attacks were of greatest severity, reaching a climax gradually and diminishing gradually thereafter. From March, 1930, to August, 1930, he was entirely free from symptoms. From August, 1930, until September 24, 1930, when he entered the hospital, he has again had pains every day, usually every three or four hours, and each attack lasts about one-half hour. These pains have been much more severe than any heretofore.

The onset of a period of attacks is usually ushered in by such prodromal symptoms as lassitude and lack of ambition; the voice becomes deeper, and he uselessly worries a great deal about trivial things. Suddenly, there will be a hot flush over the right side of the face and forehead, and swelling develops in the mucous membranes of the right side of the nose making it difficult to breathe. Soreness appears in the maxillary, orbital, temporal regions, and behind the right eye; within twenty minutes, severe sharp, shooting, knife-like pains appear in these regions. *During the pain the right eye becomes bloodshot; perspiration appears only on the right side of the forehead and face.* There is fulness and throbbing of the vessels of the forehead, eye and face on the right side only. Photophobia is associated with the seizures. The right eyelid also droops during attacks. The pulse rate drops to 45 per minute at the onset of the attack. The relief of pain is sudden and complete after one-half to one hour, and with its departure, his mental sluggishness promptly clears and his normal activities are eagerly resumed.

The pain has never extended to the left side of the face or head. There is no nausea or vomiting, no rhinorrhoea, and no scotomata or hemianopsia. There is no family history of similar attacks.

The pains have none of the characteristics of trigeminal neuralgia. They do not conform to the sensory domain of the trigeminal nerve, nor are they induced by the usual stimuli, such as cold, eating, drinking, brushing the teeth, touching the face, etc., which precipitate attacks of trigeminal tic douloureux.

The patient had a red naevus of the right eyelid, and a localized collection of large veins (suggesting an angioma) in the calf of the right leg. A congenital angiomatous mass of somewhat similar nature was suspected by Dr. Longcope and myself as perhaps existing among the sympathetic fibers along the inferior surface of the brain stem and causing his pain. It seemed clear, at least, that the patient's symptoms were referable to the sympathetic nerves of the cranium. The unilateral ptosis, sweating, photophobia, congestion of the corneal conjunctiva, swelling of the nasal mucous membrane, fulness of the vessels of the face and perhaps the bradycardia (40–50) indicated organic causation of the pain and pointed to the sympathetic nerves.

*Operation. October 28, 1930.* The stellate and inferior cervical sympathetic ganglia were removed by Adson's method, *i.e.*, after preliminary section of the second rib contiguous to the spinal column.

The patient has had no suggestion of his original pain up to the time of this writing, May 1, 1931.

*Case II.* Male, aged 51, salesman; referred by Dr. Elliot Cogswell of Hartford, Connecticut, because of excruciating paroxysmal attacks of pain in the left side of the face and head. Family history negative; no immediate or remote relatives have had any such pain. Many years ago the patient was treated for tuberculosis of the lungs and apparently cured. Past history otherwise unimportant.

During the winter of 1926, 4½ years ago, the patient developed a severe attack of neuralgia in the left side of the face. Pain persisted more or less constantly for six weeks, then disappeared. He attributed the onset of pain to exposure to cold. During the next year he had a second attack, precisely like the original seizure, in the character as well as in the distribution of the pain. It lasted three weeks. Thereafter he was perfectly free from pain or discomfort until the following year (1928). Since then attacks have been recurring every two or three months; each attack lasts two to four weeks. A diagnosis of trigeminal neuralgia had been made.

The pain is described as intense, bearing down, grinding, boring. It is invariably on the left side of the head; at no time has it ever appeared on the right side, even as a radiating overflow. The point or points of greatest intensity of the pain are variable. Most frequently it seems to be deeply in the eye or corner of the orbit. It may shift from this point and concentrate in the cheek bone, in the roof of the mouth, the tonsillar region, the supraorbital region, submaxillary

region, the temporo-mandibular joint or the parieto-occipital region. The whole left side of the head, almost exactly to the mid-sagittal line feels "sore and bruised." When the pain begins to shift from one area to another, the end of the attack is impending.

During the attacks (and sometimes between attacks) he *perspires much more freely* on the affected side. Simultaneously with the onset of pain, *the left eye becomes injected and tears flow profusely*. This is accompanied by a sensation of congestion in the nasal passages of the left side; a watery discharge from the left side of the nose quickly follows and continues throughout the attack. The watery discharge ceases after the nasal passages open as soon as the attack is over.

The pain was not confined to the sensory domain of the trigeminal nerve, but was also in the neck and back of the head. It did not radiate along the anatomical paths of the trigeminal nerve. It was not induced by the sensory stimuli (cold, rubbing the face, eating, drinking, brushing the teeth), nor did the sensory stimuli have any effect upon its intensity after its onset. The absence of all of these features, characteristic of trigeminal neuralgia, indicated that the pain had to be referred to other nerves. Finally, the pain was not of the short sharp paroxysmal character so familiar in trigeminal tic douloureux. Rather it was of gradual origin and progressive intensity and after a period of weeks (instead of minutes as obtains in trigeminal neuralgia) it subsided gradually instead of abruptly. While a gradual onset and termination are not unknown in trigeminal neuralgia, the *long duration* and the incline and decline are unknown in this condition. The positive objective features of the attacks, *i.e.*, the flow of tears, congestion of the conjunctivae, rhinorrhoea, point unmistakably to the sympathetic nerves. The diagnosis of migraine or hemicrania of sympathetic origin was therefore made upon the positive objective findings, which also indicated an organic, and eliminated a functional, origin of the attacks.

*Operation.* The patient was in an attack at the time the operation was performed, February 18, 1931: The superior cervical sympathetic ganglion was removed (avertin anesthesia). For two days the patient had no sign of his old pain, but on the third day it returned with all its characteristic features, associated also with the objective sympathetic disturbances of sweating, rhinorrhoea, photophobia and redness of the corneal conjunctiva. There was ptosis of the eyelid and narrowing of the pupil from the effects of the removal of the superior sympathetic ganglion. It was quite clear, therefore, that the removal of this ganglion not only did not cure the pain, but it did not remove all of the sympathetic nerves to this side of the head either intracranially or extracranially.

On February 25, 1931, the inferior cervical and first dorsal sympathetic ganglia were removed. Adson's procedure was used, the ganglia being reached by section of the second rib alongside the vertebral canal. There has not been the slightest suggestion of the old pain to date, May 1, 1931. A typical Horner's syndrome, of course, exists. The ptosis is perhaps slightly greater than after removal of the superior sympathetic ganglion, but this is questionable.

*Comment.* It is interesting that the removal of the superior cervical ganglion in the second case had no effect upon the patient's attacks, but that the attacks stopped instantly upon removal of the entire sympathetic supply to that side. In the light of our first experience, in which the removal of the stellate ganglion had completely removed not only the pain of similar type but also the objective evidence of function of the sympathetic fibers, it appeared probable that sympathetic fibers must enter the cranial chambers either along the sheath of the vertebral or carotid artery, or probably of both; or perhaps by fibers that pass into and up the spinal cord.

The time that has elapsed since the operation in these cases ($2\frac{1}{2}$ to $6\frac{1}{2}$ months) is too short to permit an unreserved opinion concerning the absolute value of the procedure, nevertheless the results have been striking and absolute in frequently recurring attacks. The foregoing treatment is suggested with the understanding that a greater subsequent burden of proof supported by time and additional patients rests upon the writer.

# THE EFFECT OF THE INJECTION OF URINE FROM PREGNANT MAMMALS ON OVULATION IN THE RABBIT[1, 2]

F. F. SNYDER AND G. B. WISLOCKI

From the Departments of Obstetrics and Anatomy, Johns Hopkins University

The following report consists of a brief summary of the results obtained by the injection into rabbits of urines of various pregnant animals. The results of our investigation are presented in table 1. It will be observed that the urine of pregnancy of macaque monkeys, rats, rabbits, cats and dogs was injected into mature rabbits. The specimens of urine were administered intravenously in standard doses

TABLE 1

*Negative ovulation tests in mature rabbits following injection of urines of pregnancy of various animals*

(Ten cubic centimeters of urine intravenously. Interval between injection and examination, 48 hours.)

| | | |
|---|---|---|
| Macaques | 13 tests from entire period of gestation | |
| Rats | 8 tests from entire period of gestation | |
| Rabbits | 9 tests from entire period of gestation | |
| Cats | 3 tests from middle third of gestation | |
| Dogs | 2 tests from middle third of gestation | |
| For control: | | |
| Human | 14 positives. | Diagnosis verified |
| Human | 5 negatives. | Diagnosis verified |

of 10 cc. each. Forty-eight hours were allowed to elapse before the animals were examined to see whether ovulation had occurred. The results from the urines of pregnancy of animals were controlled by the use of urines from both pregnant and non-pregnant women. It will be observed that, in contrast to positive ovulation tests produced by human urine of pregnancy, the results obtained from the urines of pregnant animals were negative.

[1] Submitted for publication May 1, 1931.

[2] This research was aided by a grant from the Linton Fund.

## DISCUSSION

These findings deserve to be examined in more detail. Urine of pregnant women contains a substance which in the immature female mouse causes ovulation or luteinization of the follicles, as Zondek and Aschheim, as well as others, have amply demonstrated. The advantages of using the rabbit, instead of the mouse, to test for substances which produce ovulation is indicated by the results of Friedman (1929) and of Schneider (1931). The rabbit does not ovulate spontaneously and if mature females are kept isolated they are excellent objects for the study of substances which produce ovulation. Inspection of the ovaries of the rabbit, one to two days after injection of the material to be tested, gives an unequivocal answer as to whether ovulation has occurred or not. The procedure in the rabbit obviates microscopic examination of the ovaries to determine the character of the reaction. Moreover, we have administered the specimens of urine intravenously as the most convenient route. Friedman (1930) has emphasized different types of reactions produced in the ovaries when urine of pregnancy is given by the subcutaneous, intraperitoneal or intravenous route. Our experience in regard to this matter is limited to two sets of experiments. In one experiment doses of 1 cc. of Parke-Davis "luteinizing hormone" (1 cc. = 50 rat units) were given to three rabbits by the intravenous, intraperitoneal and subcutaneous routes, respectively. Ovulation resulted in the rabbit receiving an intravenous injection, but did not occur in the two other animals. In a second experiment, however, in which 4 cc. were given to each of three rabbits by the three respective routes, ovulation occurred in each one of the animals. In this connection it is of interest that in several other rabbits we have observed ovulation following intravenous injection of less than 0.1 cc. of the same sample of luteinizing hormone. These experiences suggest the rôles of both route of administration and also of dosage in the effect produced in the ovaries.

Ovulation in the rabbit is clearly recognizable on gross examination of the ovaries. The day following injection of human urine of pregnancy, a crop of recently ruptured follicles is found in the ovaries. The identification of recently ruptured follicles is possible without error, by recognizing the characteristic bleeding points on the free poles of the partially collapsed follicles.

Frank ovulation has been made the sole criterion of a positive reaction in our series. Partial reactions, involving solely the presence of blood follicles or merely large follicles, have been listed as negatives. In healthy, well-nourished rabbits under experimentation, blood follicles have not been often encountered, their formation being not much more frequent than in any series of healthy untreated rabbits. In our experience with healthy animals, blood follicles have been too inconstant, and variable in their occurrence to serve as a criterion of partial reactions. Moreover, their occasional spontaneous occurrence in normal rabbits constitutes a source of error, difficult to evaluate and to separate accurately from the results of the experimental procedure. We have observed the appearance of large numbers of blood follicles accompanying, or in place of, ovulation in relatively poorly nourished animals which we kept for a while quite apart from the healthy stock of our colony. Our experience has led us to use frank ovulation as the sole criterion of deciding on a positive reaction. In the present state of inadequate knowledge of the occurrence of blood follicles, consideration of lesser reactions would give rise in such a degree to the uncertain element of subjective interpretation that the results would be ambiguous and lead to confusion.

We are reporting observations solely on the effect on the rabbit's ovaries of injecting urines from pregnant animals. We have not attempted to run parallel tests on mice or rats to see whether a given sample of urine produces equivalent results in these rodents. Instead, we are accepting as conclusive, for the urines of pregnant animals tested on mice, the data collected by Zondek (1931). It has been of interest to us to learn that the rabbit test performed with pregnant animal urines does not entirely parallel the mouse test as the latter is given in the literature.

As controls to our own series of tests on the rabbit, of urines from pregnant animals, we have used enough human urines of pregnancy to be sure of the sensitiveness of our rabbits. The human urines have proved that our rabbits have been uniformly sensitive to the substance in pregnant human urine which causes ovulation. Moreover, the diagnoses of the human urines obtained by the rabbit test were found to tally completely with the ultimate clinical findings.

We have adopted further measures to make a standard test. Ten

cubic centimeters of urine which had been filtered and, if not immediately injected, had been kept in the cold, was the standard dose for injection into the rabbit's ear-vein. The rabbits were examined 48 hours subsequent to the injection. The ovaries were exposed by a sterile operation with the animals anaesthetized with ether, amytal or avertin.

We have obtained samples of urines of pregnancy from pregnant macaque monkeys, rats, rabbits, cats and dogs. The urines from macaques have been obtained from two healthy pregnant females from Dr. Carl G. Hartman's colony. One animal was followed during the last month of pregnancy, the other was tested every few weeks during the period of gestation from the end of the first month. In the rabbit and rat a survey was made of the entire period of gestation. In rabbits, urine was collected twice or three times a week from the eighth day, or the approximate time of implantation, until the day of parturition. In the rat also the entire period of gestation was covered. In each instance the urines of three pregnant rats of the same stage of gestation were pooled and injected. Moreover, the rat urines used for each experiment represented not the urine of a single voiding, but the urine collected during an entire 6- to 12-hour period. This circumstance in the rat adds, we believe, to the value of our negative results. The urines from cats and dogs were obtained from several animals during the second third of the gestation period. The cat's and dog's urines consisted of single specimens collected by catheter.

As can be seen from table 1, our results with animal urines are negative. In adequately controlled observations on isolated mature female rabbits we have had only one exception with animal urine—a positive test in one case with rat's urine from the thirteenth day of gestation. In whatever light this positive instance may be regarded, it seems to be of little consequence, because our series shows beyond a doubt that animal urines are for all practical purposes negative as far as the rabbit is concerned. The important question, which we had originally in mind, as to whether animal urines may be used experimentally to investigate the underlying physiology of the pregnancy reaction appears to have been adequately answered in the negative.

Our results on rabbits parallel the results of the Zondek and Asch-

heim tests on mice with one interesting exception. Zondek (1931) reports that the mouse test is positive with the urines of pregnant anthropoid apes and monkeys. In contrast to his findings, we have not obtained positive results with the urine of pregnant macaques, representatives of the catarrhine monkeys. We have not had an opportunity to test the urine of any of the anthropoid apes (orang, chimpanzee, gorilla).

In keeping with our experiments with monkey urine, and contrary to Zondek's findings, Allen, Maddux and Kennedy (1931) have recently reported negative results from injecting the urine of a pregnant macaque into rats. Similarly, Gutman (1930) reports negative results in mice from transplantation of placental tissue from various animals, in comparison with positive results following implantation of human placenta.

It should be added that we have not attempted to ascertain whether urine which has been concentrated gives positive tests in the rabbit. Zondek (1931) reports, however, that concentrates of the urines of various animals tested on the mouse failed to give positive results.

From the standpoint of understanding the nature of the active principle contained in human urine, it is interesting to find tha itt is not present in animal urines. If the principle is, therefore, in its derivation a species-specific substance rather than an organ-specific one, it resembles an excretion product arising from the metabolism of pregnancy in the human rather than an active principle of general distribution and importance in mammalia. Several active extracts affecting the ovaries have been obtained from the hypophysis of various mammals without reference to pregnancy as well as from both sexes. The present substance, on the contrary, appears to differ from these, in that it occurs, so far as is known, only in the human (possibly also in certain apes) and by the fact that it is excreted during pregnancy in great quantity in the urine. Much more study will be needed before it can be said definitely what the nature and the origin of the substance occurring in human urine is. Since it is not found in animal urines of pregnancy, an important avenue for its study is closed. The fact that it is absent in animals is, however, in itself of considerable importance in defining its nature.

SUMMARY

1. The urines of pregnancy of various animals (macaque monkeys, rabbits, rats, cats and dogs) do not produce ovulation in mature, healthy, isolated rabbits.

2. The ovulation test in the rabbit does not completely parallel the similar mouse test as reported in the literature. The urines of pregnancy from monkeys and anthropoid apes have been described as giving positive tests in the mouse. In our experiments the urine from pregnant macaque monkeys has given negative tests in the rabbit. Of the two tests available for the diagnosis of pregnancy, the ovulation test in the rabbit appears to be more specific than the mouse test.

3. The observation that human urine of pregnancy produces ovulation in mature rabbits has been confirmed.

BIBLIOGRAPHY

ALLEN, E., W. P. MADDUX AND J. W. KENNEDY, 1931. Ovarian and anterior pituitary hormones from the pregnant monkey. *Proc. Soc. Exper. Biol.*, vol. 28, p. 403.

FRIEDMAN, M. H., 1929. Mechanism of ovulation in the rabbit. II. Ovulation produced by the injection of urine from pregnant women. *Am. Jour. Physiol.*, vol. 90, p. 617.

*Idem.*, 1930. Humoral mechanisms concerned in ovulation in the rabbit. *Endocrinology*, vol. 14, p. 328.

GUTMAN, M., 1930. Untersuchungen über das Hypophysenvorderlappenhormon bei trächtigen Säugetieren. *Arch. f. Gyn.*, Bd. 14, S. 22.

SCHNEIDER. P. F., 1931. A hormone test for the diagnosis of early pregnancy. *Surg. Gyn. & Obst.*, vol. 52, p. 50.

ZONDEK, B., 1931. Die Hormone des Ovariums und des Hypophysenvorderlappens. Anhang I. Die hormonale Schwangerschaftsreaktion aus dem Harn bei Mensch und Tier. Julius Springer, Berlin.

# A HUMAN TUBAL EGG, UNFERTILIZED[1]

WARREN H. LEWIS

*From the Department of Embryology, Carnegie Institution of Washington, and The Johns Hopkins Medical School, Baltimore, Md.*

On February 4, 1931, Dr. Thomas S. Cullen of the Johns Hopkins Hospital very kindly sent to our laboratory a large myomatous uterus with attached tubes from a hysteromyomectomy and bilateral salpingectomy. The patient was an unmarried negress, 40 years of age, and her last menstrual period had begun on January 14, 21 days before the operation. Previous to the present illness the menstrual periods had been at 28-day intervals and of 3 to 4 days' duration with moderate flow. Since about a year the menstrual flow had been more profuse and of longer duration, and the one of January 14 had lasted about two weeks. The previous menstrual period had begun December 10, 1930. Thus there was an interval of 35 days between the onset of the previous period and that of the last one on January 14th.

If ovulation occurred from the (11th or) 12th to the 14th days of the cycle (Allen, Pratt, Newell and Bland), this egg would be from 7 to 10 days old. One would expect an egg of this age, however, to be in the uterus, if the rate of descent through the tube corresponds with that of other mammals. In the mouse, rat, guinea-pig, rabbit, sheep and cow, the fertilized, unfertilized and abnormal eggs descend in the tubes at about the same rate and enter the uterus during the early part of the fourth day. If somewhat the same conditions prevail for man, this egg, which was still in the tube, could not be much over three days old and consequently ovulation could not have occurred until about 18 days after the onset of the last menstrual period. It may be that human eggs do not enter the uterus as rapidly as do those of other mammals, or the myomatous condition of the uterus may have delayed passage into the uterus. There did not appear, however, to be any obstruction to the tube.

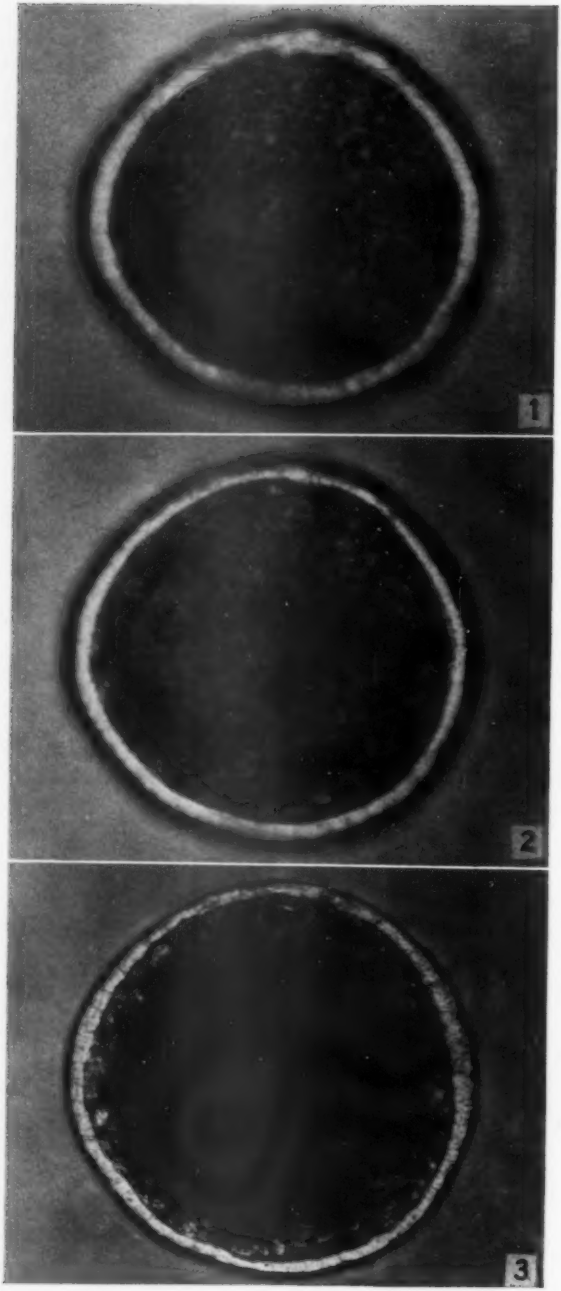
[1] Submitted for publication May 1, 1931.

It has already been noted that the previous cycle was lengthened out to 35 days and if the one at the time of the operation had been destined to have a similar length, our estimated time of ovulation, namely, 18 days after the onset of the last menstrual period, would bring the time of ovulation at the middle of the cycle.

The tubes were cut off flush with the wall of the uterus and each tube was washed out with about 5 cc. of Locke solution into two salt-cellars with round inside bottoms. The egg which quickly settled to the bottom was found in the first dish of washings from the right tube. The egg was transferred from the salt-cellar with a small pipette to a hanging drop of Locke solution and the photographs shown in figures 1, 2 and 3 were taken while it was still in the fresh and perhaps living condition.

The egg was not quite spherical. Its outside diameters as measured from the photographs were 145 and 151 micra. The inside diameters of the zona, equivalent to the outside diameters of the vitellus, were 133 and 138 micra. The mean outside diameter was about 148 micra and the mean diameter of the vitellus about 136 micra. This vitellus is somewhat larger than most other mammalian tubal eggs and considerably larger than the vitelli of the tubal eggs of Allen, Pratt, Newell and Bland, which averaged about 109 micra in diameter. These vitelli had evidently undergone shrinkage, because in freshly ovulated eggs they completely fill the zona. The mean inner diameters of the zonas of their eggs I and III were 135 and 120 micra, respectively. These latter figures indicate more nearly the diameters of the vitelli of freshly ovulated eggs. Table I gives the average inner diameter of the zonas (equivalent to the diameter of the vitelli of freshly ovulated eggs) of several mammalian eggs. It indicates that there may be some correlation between the size of the mammal and the size of the egg.

The vitellus of our tubal egg was rather dark and was everywhere in contact with the inner surface of the zona. Its surface was coarsely granular in appearance as shown in figures 1, 2 and 3. The coarsely granular bodies appeared to be yolk. There were three types of yolk bodies. The largest ones, greenish-yellow in color, were just beneath the smaller superficial large white yolk bodies. The yellow yolk bodies or spheres varied from 12 to 16 micra in diameter and were seen


1
2
3

as large dark bodies in the photographs. They were irregularly distributed. The exact number was uncertain but 20, including some rather hazy ones, were counted. They were not homogeneous but contained small fat-like globules embedded in a homogeneous matrix.

TABLE I

*Measurements from photographs (Carnegie Collection) of early mammalian tubal eggs in the living state*

Inner diameter of zona in micra

| ANIMAL | STAGE | NUMBER OF EGGS | AVERAGE DIAMETER | OBSERVER |
|---|---|---|---|---|
| Mouse | 1c | 4 | 90 | Lewis |
| Guinea-pig | 1c | 1 | 93 | Squier |
| Guinea-pig | 2c | 3 | 96 | Squier |
| Dog | 1c | 3 | 120 | Hartman |
| Rabbit | 1c | 2 | 128 | Gregory |
| Rabbit | 2c | 4 | 127 | Gregory |
| Monkey | 2c | 1 | 127 | Lewis and Hartman |
| Pig | 1c | 4 | 130 | Heuser and Streeter |
| Cow | 2c | 1 | 135 | Hartman, Lewis, Miller and Swett |
| Man | 1c | 1 | 136 | Lewis |

With a Wrattan-Wainwright C (blue) filter they were of a deep violet color and stood out very clearly from the rest of the vitellus as very dark bodies. The white yolk spheres, closely packed together at the periphery of the vitellus, formed a sort of a mosaic which was almost cellular in appearance where they were flattened somewhat against the

FIG. 1. SURFACE VIEW OF THE UPPER POLE OF THE EGG; MOSAIC-LIKE APPEARANCE OF THE WHITE YOLK SPHERES

Ten yellow yolk sphere shadows are to be seen, some of them rather dimly. × 405 dia.

FIG. 2. A SOMEWHAT DEEPER FOCUS SHOWING 4 OF THE YELLOW YOLK SPHERES ABOUT IN FOCUS AND THE MOSAIC OF WHITE YOLK SPHERES

× 405 dia.

FIG. 3. MEDIAN FOCAL PLANE

The radial structure of the zona is indicated in places. Peripheral layer of white yolk spheres against the inner wall of the zona. Some of the dark yellow yolk spheres can be seen just beneath the peripheral layer of white yolk spheres. × 405 dia.

zona. They were from 6 to 10 micra in diameter and contained fat-like globules embedded in a homogeneous matrix. Between the white yolk spheres were numerous very small fat-like globules.

The surface layers of the vitellus were so light-refracting that it was impossible to obtain an adequate idea of the interior of the egg.

The optical equatorial plane of the zona (figure 3) shows a very distinct radial striation indicating the position of the zonal processes of the coronal cells, which are supposed to pierce the zona and come into contact with the vitellus when the coronal cells are in position. The zona in this optical section varies from 6 to 11 micra in thickness.

Since this unfertilized egg was several days old, it was probably somewhat different in appearance from a freshly shed one. Some freshly ovulated mammalian eggs (guinea-pig and dog) contain many rather large yolk spheres which give the egg a dark appearance. Sections of the fixed pig eggs (Heuser and Streeter) show that in this egg also there are many large yolk spheres. The yolk spheres in the mouse egg are intermediate in size. Those in the rabbit are still smaller, while the monkey egg has a very fine granulation and here the nucleus is easily recognized.

## BIBLIOGRAPHY

ALLEN, PRATT, NEWELL AND BLAND, 1930. Human tubal ova; related early corpora lutea and uterine tubes. *Contributions to Embryology*, Vol. 22, pp. 45–76.

GREGORY, P. W., 1930. The early embryology of the rabbit. *Ibid.*, Vol. 21, pp. 141–168.

HARTMAN, C., 1929. How large is the mammalian egg? *Quarterly Review of Biology*, Vol. 4, pp. 373–388.

HARTMAN, LEWIS, MILLER AND SWETT, 1931. First findings of tubal ova in the cow, together with notes on oestrus. *Anat. Rec.*, Vol. 48.

HEUSER, C. H. AND G. L. STREETER, 1929. Early stages in the development of pig embryos from the period of initial cleavage to the time of the appearance of limb buds. *Contributions to Embryology*, Vol. 20, pp. 1–30.

LEWIS, W. H., 1931. An unfertilized human tubal egg. *Anat. Rec.*, Vol. 48, Suppl. p. 52 (*Proc. Anat. Soc.*).

# THE MECHANISM OF THE KETOGENIC DIET IN EPILEPSY[1,2]

EDWARD M. BRIDGE AND L. V. IOB

*From the Department of Pediatrics, Johns Hopkins University and the Harriet Lane Home of the Johns Hopkins Hospital*

Although the value of the ketogenic diet in the treatment of epilepsy in childhood can hardly be questioned, the mechanism by which it produces its beneficial effect has not been explained. Investigators of the problem have hoped that if the therapeutic principle of such a diet were better understood, it might be possible to overcome some of the objectionable features of the régime without losing the effectiveness, and perhaps even to make it successful in patients who now fail to respond. The following report is presented as a contribution toward a better understanding of the action of the diet.

## SUGGESTED THEORIES

Early investigators of the action of the diet advanced the theory that the ketosis accompanying both starvation and the high fat diet offered adequate explanation for the beneficial effects of such régimes on the course of epilepsy. For a time it was considered only necessary to establish a strong ketosis in order to obtain good results. Mild degrees were advocated in mild cases, severe degrees for the more refractory patients. In support of this theory instances were described in which the improvement of epileptic seizures seemed to correspond to the severity of the ketosis. Several reports have been made, most recently that of Helmholz and Keith (1), indicating that the ketone bodies contain certain anaesthetic properties. The administration of glucose overcomes these effects by destroying the ketones. Gradually, however, the exceptions to this hypothesis have become more

[1] This work was made possible by a grant from the Epilepsy Medical Research Fund.

[2] Submitted for publication April 20, 1931.

and more apparent, for frequently no improvement results in spite of severe ketosis, and at times good results are obtained without the formation of ketone bodies. A quantity of sodium bicarbonate, insufficient to produce definite alkalosis, may be added to the high fat diet with a marked increase in the excretion of ketones and yet the frequency of the attacks is increased. A comparison of the degree of ketosis seen in epileptic patients on a ketogenic diet with the amount of ketone bodies needed to produce intoxication in animals, makes it unlikely that the whole effect of the diet is to be accounted for in this way. Although at times there has appeared to be a general inverse relationship between the frequency of the seizures and the concentration of ketones in the blood, urine, and expired air, it has been impossible to establish any constant correlation. At present it is generally admitted that the parallelism which seems to exist is not one of cause to effect, but more likely indicates the action of some other factor which is usually, but not necessarily, associated with the ketosis.

Numerous studies have been made attempting to correlate changes in the chemical composition of the blood with epileptic seizures. Sugar, uric acid, chlorine, phosphorus, calcium, fixed base, etc., have received attention without adding anything of importance to an understanding of the diet.

Several investigators have maintained that the epileptic seizure was dependent on a temporary wave of alkalosis in the body. As a corollary to this idea others have suggested that the acidosis which accompanies both starvation and the high fat diet may be the beneficial factor in the improvement in epileptic seizures which follows such treatment. Strong evidence in support of this suggestion is found in the direct relationship which seems to exist in some patients between the carbon dioxide combining power of the blood and the epileptic seizures. Also, a simple acidosis, resulting from the ingestion of inorganic acids, has been shown to cause striking improvement in certain patients often as satisfactory as that resulting from starvation. But here again the exceptions have overbalanced the rule. In the first place, the improvement following acidosis produced by the ingestion of inorganic acids is only temporary, in spite of the fact that the carbon dioxide combining power of the blood remains low. Secondly, the acidosis seen during the first days of fasting or dietary treatment

often disappears after a few weeks although the improvement remains. Again, the production of a marked alkalosis in epileptic patients is not necessarily associated with a return of seizures. And finally, more recent careful studies have failed to establish any direct relationship of blood acidity to seizures. As with ketosis, it is now becoming generally admitted that while the beneficial effects of the ketogenic régime may seem to parallel the degree of acidosis they are not entirely dependent on it.

Fay (2), in 1929, and later in the same year McQuarrie (3) pointed out the importance of changes in water metabolism to the course of epilepsy, and suggested that the action of the ketogenic diet might be explained on this basis.

TABLE I*

*Balance studies on normal individual showing effect of a sudden increase of fat in the diet*

| | GAINS OR LOSSES OF BODY MATERIAL | | | | | | GAIN OR LOSS BY BALANCE |
|---|---|---|---|---|---|---|---|
| | Protein | Fat | CHO | $H_2O$ | Ash | Total | |
| | gm. | gm. | gm. | gm. | gm. | gm. | gm. |
| *CHO diet:* P 101, F 115, CHO 730— average of 3 days | −5 | −25 | −50 | +165 | +3 | +88 | +61 |
| *Fat diet:* P 103, F 302, CHO 320— average of 3 days | −12 | −47 | −3 | −906 | −7 | −947 | −913 |

* Benedict and Milner, 1907.

Several decisive experiments have been reported during the past forty years which have demonstrated that changes from a high carbohydrate to a high fat diet may be associated with a marked loss of body-weight, which is accounted for, not as true tissue destruction, but as a fluid loss. The experiment of Benedict and Milner published in 1907 shows this fact most clearly (4). While the diet used in the experiment was high in fat, the ketogenic threshold was not exceeded. Table I, which is derived from their report, shows the results obtained on a normal individual who lived for several days under the carefully controlled conditions of a metabolism room, performing a constant amount of work each day. On a diet high in carbohydrate he gained weight very slightly. When the same number of calories was sup-

plied largely in the form of fat, he lost weight rapidly, the loss being accounted for by a water loss.

If a child who has previously been on a low carbohydrate feeding is suddenly given a large proportion of carbohydrate foods, weight gains are seen to follow so rapidly that they can only be accounted for as water gains. This effect, produced by the reversal of the procedure mentioned in the preceding experiment, has been recognized for years

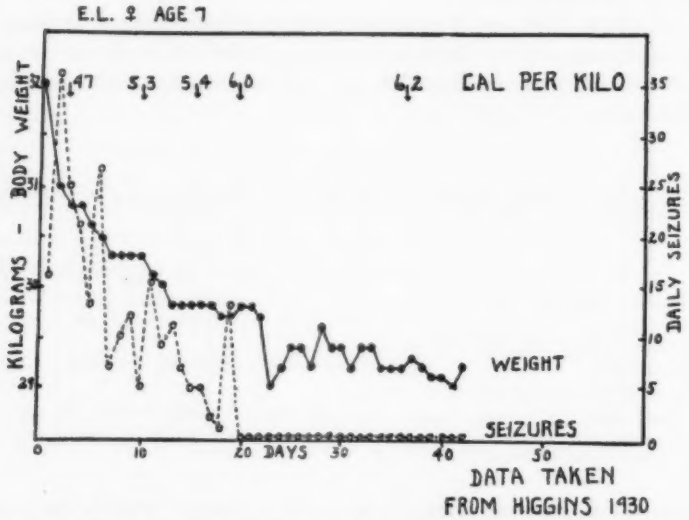

E.L. ♀ AGE 7
4.7 5.3 5.4 6.0 6.2 CAL PER KILO
KILOGRAMS - BODY WEIGHT
DAILY SEIZURES
WEIGHT
SEIZURES
0 10 20 DAYS 30 40 50
DATA TAKEN FROM HIGGINS 1930

CHART 1. BODY-WEIGHT AND EPILEPTIC SEIZURES IN PATIENT DURING THE GRADUAL INSTITUTION OF A KETOGENIC DIET

in the growth of infants. In all probability the biochemical nature of the two phenomena is fundamentally the same.

That the same principles hold in relation to the ketogenic diet and seem to bear a relationship to the course of the epilepsy can be visualized in the prompt and sharp reaction of certain patients following the institution of the diet. Chart 1 is made from data reported by Higgins (5) concerning an eight-year-old child having frequent petit mal seizures daily. This child was placed on a diet, the fat proportion of which was gradually increased. The decrease in the number of daily seizures closely parallels the weight loss. When the weight became

stationary at a new level, no further attacks developed. In the two years which have elapsed since then the child has remained free from seizures and the weight loss has been very slowly regained as normal growth.

## PLAN OF STUDY

In 1923 Gamble, Ross and Tisdall published their work on "The Metabolism of Fixed Base During Fasting," in which they showed that the rapid loss in weight seen during the early days of fasting does not represent true tissue destruction but is largely accounted for as a loss of water from the body (6). Furthermore, this water is apparently bound to a definite amount of base, for on the basis of the sodium, potassium, and nitrogen excreted in the urine during fasting these investigators were able to calculate the water losses which corresponded to the weight losses. Knowing that sodium represents nearly all the base of the extracellular fluids of the body, while potassium is the chief base of the tissues, it was possible to calculate with a fair degree of accuracy the source of the fluid losses. In this way it was demonstrated that the rapid loss in weight during the early days of fasting is accounted for in large part as extracellular (blood serum, lymph, etc.) and intracellular fluid (tissue juices).

In order to test the hypothesis that improvement from the ketogenic diet parallels the tendency to remove fluid stores from the body, three pairs of children were selected for study, in such a way as to make comparison of one with the other as reliable as possible. All were girls between the ages of eight and thirteen having frequent petit mal seizures daily. No evidence of organic brain disease was found. All were fasted five or six days, distilled water only being allowed by mouth. A cleansing tap-water enema was administered on the first morning of the fast; no stools were passed thereafter until the diet was instituted. The urine was accurately collected in 24-hour specimens and analyzed for nitrogen, sodium, potassium, chloride, phosphate and sulphate.[3] The two girls in group (1) had been previously untreated;

[3] Total nitrogen was determined by the macro-Kjeldahl method; sodium by the Barber-Kolthoff method (7), as modified by Drake (unpublished); potassium by an unpublished method of Fiske; chloride by the method of Fiske and Lin (8); phosphate by the method of Fiske and Subbarow (9); and sulphate by the Fiske method (10). All determinations were done in duplicate.

TABLE II

*Data obtained from fasting epileptic children. Urine collected in 24-hour specimens*

| CASE | AGE | GROUP | WEIGHT | DAY OF FAST | WEIGHT LOSS | EXCRETION IN URINE—CC. N/10 | | | | | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | K | Na | Cl | $PO_4$ | $SO_4$ | |
| | | | *kg.* | | *kg.* | | | | | | *gm.* |
| K. H. | 13 | (1) | 36 | 1 | 1.0 | 325 | 506 | 600 | 267 | 165 | 5.3 |
| | | | | 2 | 1.3 | 994 | 891 | 284 | 899 | 517 | 14.7 |
| | | | | 3 | | | | | | | |
| | | | | 4 | 0.2 | 336 | 255 | 132 | 499 | 241 | 7.8 |
| | | | | 5 | 0.35 | 153 | 32 | 107 | 328 | 183 | 5.8 |
| E. W. | 13 | (2) | 38 | 1 | 0.5 | 201 | 411 | 348 | 222 | 53 | 3.7 |
| | | | | 2 | 1.9 | 822 | 1,384 | 446 | 704 | 222 | 8.6 |
| | | | | 3 | | | | | | | |
| | | | | 4 | 0.4 | 390 | 240 | 210 | 427 | 175 | 5.0 |
| | | | | 5 | 0.4 | 318 | 81 | 213 | 458 | 203 | 5.4* |
| M. S. | 12 | (3) | 36 | 1 | 0.5 | 207 | 93 | 322 | 299 | 138 | 4.8 |
| | | | | 2 | 0.9 | 531 | 212 | 230 | 559 | 233 | 8.6 |
| | | | | 3 | | | | | | | |
| | | | | 4 | 0.0 | 243 | 67 | 71 | 296 | 132 | 4.3 |
| | | | | 5 | 0.1 | 235 | 75 | 100 | 422 | 196 | 6.6 |
| L. D. | 7 | (1) | 28 | 1 | 0.4 | 153 | 84 | 195 | 52 | 32 | 1.2 |
| | | | | 2 | 1.0 | 351 | 416 | 91 | 400 | 185 | 5.3 |
| | | | | 3 | 0.7 | 323 | 287 | 99 | 318 | 136 | 4.5 |
| | | | | 4 | 0.6 | 362 | 212 | 116 | 458 | 202 | 6.4 |
| | | | | 5 | | | | | | | |
| G. H. | 10 | (2) | 33 | 1 | 1.5 | 360 | 906 | 1,077 | 162 | 79 | 3.2 |
| | | | | 2 | 0.6 | 238 | 349 | 176 | 226 | 100 | 3.8 |
| | | | | 3 | 0.6 | 302 | 250 | 73 | 276 | 117 | 3.8 |
| | | | | 4 | 0.3 | 347 | 209 | 65 | 325 | 194 | 4.4 |
| | | | | 5 | 0.6 | 441 | 79 | 137 | 520 | 315 | 8.1 |
| | | | | 6 | | | | | | | |
| L. D. | 8 | (3) | 28 | 1 | 0.6 | 164 | 245 | 272 | 228 | 81 | 3.4 |
| | | | | 2 | 0.5 | 132 | 140 | 68 | 122 | 48 | 2.0 |
| | | | | 3 | 0.2 | 204 | 185 | 77 | 226 | 122 | 4.1 |
| | | | | 4 | 0.3 | 195 | 113 | 57 | 224 | 110 | 3.6 |
| | | | | 5 | 0.4 | 89 | 41 | 33 | 112 | 53 | 1.7 |

* Estimated.

both became free from seizures during the fast. The two children representing group (2) had received a high ratio ketogenic diet (4 grams of fat to 1 gram of carbohydrate plus protein—FA:G ratio 3.5:1) for at least nine months without benefit; one of these patients was rendered seizure-free by the fast, while the attacks of the other were too infrequent to judge. Group (3) had received the same diet for at least six months with marked improvement in seizures. One of the latter had been without seizures during the whole period; the attacks of the other had been reduced from 12 to 20 a day to one or occasionally two. Starvation made no change in their condition. All the patients of groups (2) and (3) who had been previously on the diet showed good ketosis, and at least during the early part a moderate acidosis. The results are shown in Table II.

During fasting the body receives its energy through the destruction of muscle tissue and fat. The amount of muscle destroyed can, of course, be calculated from the urinary excretion of nitrogen. The amount of water and electrolytes derived from the consumption of fat is negligible. From analyses of muscle it has been determined that fairly definite amounts of water and base are bound with the protein, so that the nitrogen excretion being known, the sodium and potassium lost because of the destroyed muscle can be calculated. When this is deducted from the total excretion of these substances, a considerable excess is left unaccounted for, which, according to Gamble, Ross and Tisdall, corresponds to the water losses over and above those due to tissue destruction and, therefore, representing actual dehydration. The methods of calculation used by these authors have been followed here.[4]

## RESULTS

The daily potassium losses during fasting, unaccounted for by the destruction of protein, in these three groups of children are shown in Charts 2 and 3 in terms of cc. N/10 K lost daily. While there are slight differences between the groups, the general course of the curves

[4] Gamble, Ross and Tisdall use the following figures as the basis of their calculations:

| | |
|---|---|
| Nitrogen in muscle tissue | 3.4 per cent |
| Water in muscle tissue | 76 per cent |

is the same regardless of the previous diet or of improvement during the fast.

In this connection it is of interest to recall the work of Gamble and Hamilton (11) on the fluid excretion of epileptic children as influenced by a series of convulsions. They studied the acid-base balance of an epileptic child who was subject to "recurring periods of severe seizures, lasting each time about two days, with intervals between of four to seven days." The diet and fluid intake were held constant. Although during the days of seizures a marked increase occurred in the output

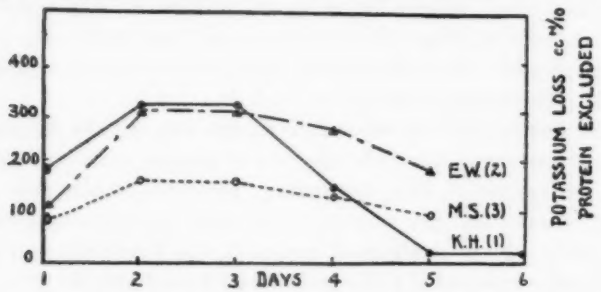



CHART 2. Daily loss of potassium unaccounted for by tissue destruction during fasting; (1) normal diet previously; (2) ketogenic diet previously, no beneficial effect; (3) ketogenic diet previously with marked improvement in epilepsy. All patients were girls 12 to 14 years of age weighing 36 to 38 kilograms.

of fluid and fixed base, the potassium excretion remained relatively constant throughout. Discussing these results in relation to the studies of Gamble, Ross and Tisdall (1923) on fasting epileptic chil-

---

Potassium per gram muscle water..... 1.08 cc. N/10
Sodium per gram muscle water..... 0.458 cc. N/10

Urine nitrogen $\times \frac{100}{3.4}$ = grams muscle tissue destroyed

$N \times \frac{100}{3.4} \times 0.76$ = grams water derived from the destroyed muscle

$N \times \frac{100}{3.4} \times 0.76 \times 1.08$ = cc. N/10 K derived from the destroyed muscle

$N \times \frac{100}{3.4} \times 0.76 \times 0.458$ = cc. N/10 Na derived from the destroyed muscle

dren, they were led to conclude that the intracellular fluid lost during fasting was not of therapeutic significance.

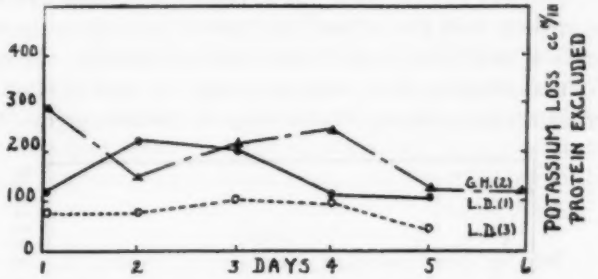



CHART 3. Daily loss of potassium unaccounted for by tissue destruction during fasting; (1) normal diet previously; (2) ketogenic diet previously, no beneficial effect; (3) ketogenic diet previously with marked improvement in epilepsy. All patients were girls 7 to 10 years of age weighing 28 to 33 kilograms.

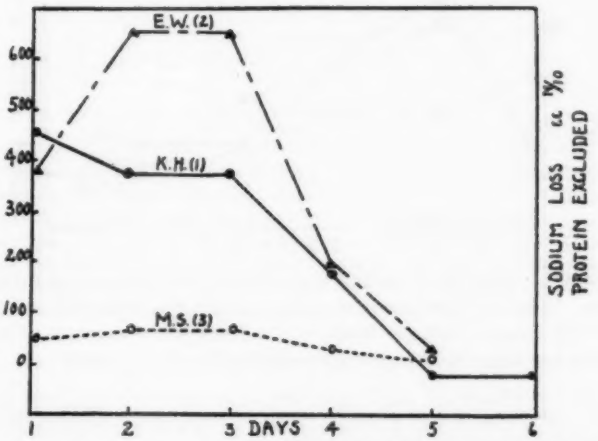



CHART 4. Daily loss of sodium unaccounted for by tissue destruction during fasting; (1) normal diet previously; (2) ketogenic diet previously, no beneficial effect; (3) ketogenic diet previously with marked improvement in epilepsy. All patients were girls 12 to 14 years of age weighing 36 to 38 kilograms.

Charts 4 and 5 show the sodium losses unaccounted for by the destruction of protein. Whereas the group (2) which had been unim-

proved by the diet alone lost approximately as much sodium as the untreated group (1), the children who had been much improved by the diet (3) lost very little sodium. Since this sodium is assumed to come almost entirely from the extracellular fluid of the body, a curve of the extracellular fluid losses would be approximately parallel. It should be noted in confirmation that, whereas groups (1) and (2) lost weight rather precipitously during the first days of the fast, group (3) lost a

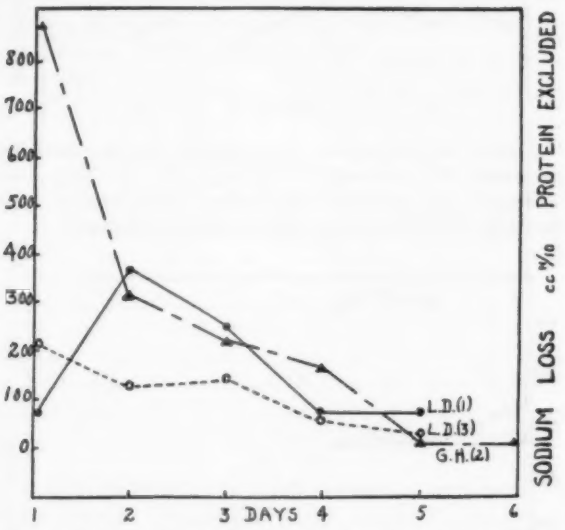



CHART 5. Daily loss of sodium unaccounted for by tissue destruction during fasting; (1) normal diet previously; (2) ketogenic diet previously, no beneficial effect; (3) ketogenic diet previously with marked improvement in epilepsy. All patients were girls 7 to 10 years of age weighing 28 to 33 kilograms.

smaller amount rather steadily, and the excretion of the various elements was comparatively constant.

It appears, therefore, that if the ketogenic diet alone has succeeded in removing the "surplus" sodium from the body, improvement has resulted, and starvation contributes nothing further. But when the diet has failed to do this even in the presence of good acidosis and ketosis, no improvement has resulted. In the latter case fasting may

bring about the same sodium losses as if no special diet had been given, and under these circumstances improvement may follow. Table III summarizes the result.

### FASTING AS A DEHYDRATING MEASURE

If this reasoning is correct, any changes that can be made in the ketogenic régime which will help in removing the excess sodium and extracellular fluid from the body would be valuable adjuncts to the treatment. Several methods are known, of which the most studied

TABLE III*

*Summary of the relation of fasting to previous dietary treatment in epileptic children*

| GROUP | | PREVIOUS TREATMENT | | EFFECT OF FAST ON | |
|---|---|---|---|---|---|
| | | Nature | Result | Sodium excretion | Seizures |
| 1 | K. H. | 0 | 0 | ++++ | ++++ |
| | L. D. | 0 | 0 | +++ | ++++ |
| 2 | E. W. | Fat diet | 0 | ++++ | ++++ |
| | G. H. | Fat diet | 0 | ++++ | ? |
| 3 | M. S. | Fat diet | +++ | + | 0 |
| | L. D. | Fat diet | ++++ | ++ | 0 |

The difficulties encountered in attempting to conduct such an investigation as the present have made it necessary to study thoroughly a limited number of patients, rather than a larger series of children on a statistical basis. The results have seemed so definite as to justify their presentation here. However, too general conclusions should not be drawn, especially as to the nature of the epileptic seizure. The only apparent exception to the results obtained in these groups of children to a period of fasting has been in another child in whom the experimental conditions were quite different from the above.

has been starvation. If we consider fasting from the viewpoint of nutrition and metabolism, it is evident that it represents for the organisms not only a high fat diet (body fat and protein are being utilized) but also an ash-free diet as far as intake is concerned. Ketosis and consequent acidosis are superimposed. It has been shown in the above discussion that the fat diet alone may produce this loss of fluid. The question might be raised as to whether the other factors also may not have a similar action.

To test out this possibility a normal adult fasted three days, and

over a later period lived on an ash-free diet which was normal in the proportion of food elements. Determinations were made on the urine as with the fasting epileptic children. Table IV shows the results. Both the weight losses and sodium losses correspond closely in both experiments, showing that a simple ash-free diet without the other factors of starvation may produce similar changes. To carry the experimet still further a normal adult lived for three days on a sodium-free diet, and another on a chlorine-free diet. The former lost both weight and sodium in large amounts, while the latter lost less weight and practically no sodium. This would suggest that the loss of body water resulting from a period of starvation may be dependent to some extent on the failure of the body to receive the necessary electrolytes, particularly sodium.

TABLE IV

*Extracellular fluid losses from normals (cc.)*

| DIET | CASE | WEIGHT | FIRST DAY | SECOND DAY | THIRD DAY | TOTAL | WEIGHT LOSS |
|---|---|---|---|---|---|---|---|
| | | *kg.* | | | | | *kg.* |
| Starvation | E. B. | 58 | 417 | 233 | 61 | 711 | 2.93 |
| Ash-free diet | E. B. | 58 | 566 | 330 | | 896 | 1.97 |
| Sodium-free diet | R. M. | 65 | 663 | 139 | 85 | 887 | 1.90 |
| Chlorine-free diet | N. C. | 82 | 14 | 114 | +38† | 90 | 1.30 |
| Normal diet + $CaCl_2$ | B. K.* | 25 | 266 | 244 | 57 | 567 | |

* Gamble, Blackfan and Hamilton, 1925.

† Indicates apparent retention.

One other observation is of interest in this connection, viz. that reported by Gamble, Blackfan and Hamilton on the diuretic action of acid-producing salts (12). These workers produced an acidosis in a normal child by administering calcium chloride. A loss of body-weight resulted coincidently with the excretion of a definite excess of water and sodium in the urine. When considered on the basis of body-weight the amount of sodium lost under these conditions is of the same order of magnitude as the losses found in the above-mentioned adults.

Thus we see that during complete starvation at least three factors are operating to remove sodium and extracellular fluid from the body: (1) a high fat diet, (2) lack of sodium intake and, (3) an acidosis. At present there is no evidence to indicate that ketosis has or has not a

similar action. The temporary improvement associated with a simple acidosis in certain epileptics has been mentioned above. It is of interest in this connection that Bartlett, Metcalf and Fox have recently reported improvement in patients with epilepsy using diets low in ash (13). On theoretical grounds it seems likely that the rigid restriction of fluids now being prescribed for epilepsy would also deplete the body reserves of extracellular fluid and produce an accompanying loss of sodium.

## DISCUSSION

In all probability the effect of the fat diet *per se* on the body fluids and the epilepsy is much more important than the acidosis or lack of sodium. Rarely will the addition of inorganic acids to a ketogenic diet improve a patient with epilepsy more than the diet alone; nor will the increase or decrease of sodium in the diet have an appreciable effect. However, as mentioned above, sodium bicarbonate may increase the ketosis and cause a return of seizures even though the carbon dioxide combining power of the blood is not raised above normal. The explanation of this phenomenon is probably similar to that given by Joslin (14) in accounting for the edema encountered so frequently in diabetics after the administration of sodium bicarbonate. He feels that such edema is associated with a retention of sodium chloride, and can be eliminated by reducing the salt in the diet. One epileptic patient under our observation was weighed before her breakfast and supper while taking a ketogenic diet. Under ordinary conditions her evening weight was 100 to 300 grams above her morning weight. Under a heavy dose of sodium bicarbonate the fluctuation became much greater, amounting to 700 to 1200 grams. On these days of great fluctuation, although the degree of ketosis was nearly doubled, the frequency of seizures increased considerably (Chart 6). The experiment suggests that the known ill effects of alkali in epilepsy may be associated with an action on the body fluids rather than any change in the acidity of the body. Such a view is in accord with the explanation of the beneficial action of a simple acidosis mentioned above.

From his experience with the dehydration treatment of epliepsy Fay has been led to conclude that the mechanism responsible for the grand mal convulsion is fundamentally different from that of the petit

mal, for while this form of treatment may prove entirely satisfactory for the former, it rarely improves the latter; in fact dehydration frequently makes patients with petit mal seizures decidely worse. It may also transform a major type of epilepsy into a minor one. It

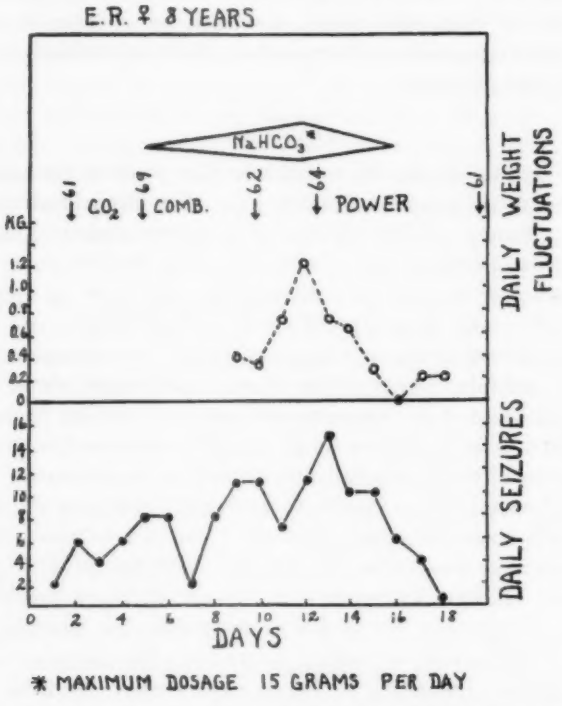



CHART 6. RELATION OF EPILEPTIC SEIZURES TO DAILY FLUCTUATION IN BODY-WEIGHT IN PATIENT WHO WAS GIVEN SODIUM BICARBONATE WHILE ON A KETOGENIC DIET

seems more likely to us that the difference in response of the two forms of the disease to fluid restriction need not be interpreted as a difference in etiology but could be explained as well by secondary reactions. We have had occasion to observe a child whose petit mal seizures were greatly increased in frequency by a week of severe water restriction,

with marked improvement when fluids were again allowed liberally. Although she lost a moderate amount of weight during the period of fluid restriction it was not accompanied by a loss of equivalent base in the urine. Possibly the symptoms may be accounted for by an actual concentration of electrolytes in the body with the poor circulation which is known to accompany such a state.

The reverse of this phenomenon, that of overhydration, also occurs. The petit mal seizures of another child were definitely more frequent when he was on a practically ash-free diet. The excretion of base in his urine was more than could be accounted for by a small weight loss. This suggested that a state of overhydration may have existed, a condition which has been known for years to increase the susceptibility to epileptiform attacks.

It seems obvious that the mechanism of the seizure and its control is far from being a simple one; nor is the dehydrating effect of the various forms of treatment of simple, easily understandable action. Rather the problem takes on additional complications with each new advance in our understanding of it. While forms of treatment which tend to remove body fluids seem to be of distinct value in epilepsy, if the body is not left in a reasonably normal state of hydration, more harm than good may result. The nature of the fluid removed appears to be influenced by various factors, which also determine whether the removal will be beneficial or detrimental. A great deal of careful work is needed to place the problem on a firmer basis.

Concerning the management of the ketogenic diet several points are of interest. Obviously tests for ketosis and acidosis are of little aid in determining whether or not the diet is effective to the full extent of its possibilities. Strongly positive reactions for both may be present and yet the fluid relations of the body be undisturbed until a period of starvation is superimposed. Possibly weight changes might be utilized as a more valuable indicator. Consequently, therefore, no ketogenic diet can be considered a failure unless it has been instituted following a fast, and has then failed to maintain the good effects of fasting. Although in a certain proportion of patients the diet is effective without the preliminary starvation, in others it is not. The original plan of Peterman recommending the fasting period before instituting the

high fat diet is thus more sure to yield results than the later routine of the gradually increasing proportion of fat in the diet.

The preliminary period of starvation has been used by some workers simply as a means of adjusting the patient's tastes and intestinal tract to the disagreeable diet to follow. They usually allow water, broth, and in some instances orange juice in liberal amounts. The fast advocated usually lasts 2–3 days. From the foregoing discussion it is plain that the fast has a much more important function than this. Five days without food is a minimum for reducing the body fluids to the new level. Water should be encouraged up to a liter a day, but the use of broth and orange juice, except when complications develop, tends to counteract some of the valuable effects of the fast.

## SUMMARY

Evidence is presented showing that neither the ketosis nor the acidosis accompanying the so-called ketogenic treatments for epilepsy can be considered the sole beneficial factor of such régimes.

Experiments are reported showing that if the high fat diet has succeeded in removing a surplus of extracellular fluid from the body, improvement in seizures is associated; if the diet has not removed this fluid, it has not affected the course of the disease.

Since a simple acidosis, and a sodium-free diet also tend to produce similar losses of fluid from the body, fasting may be considered a triple mechanism—high fat diet, sodium-free diet, and acidosis—for the removal of extracellular fluid, while the ketogenic diet has only two factors—high fat diet, and acidosis.

Since no ketogenic diet can be considered a failure until it has failed to maintain the good effects of fasting, it is believed that the institution of the high fat diet should always be preceded by a minimum of five days of complete starvation, water only being allowed.

Although the data here presented have been obtained from children with petit mal epilepsy, there is no apparent reason to suppose that they do not apply to children with major convulsions as well.

## BIBLIOGRAPHY

(1) *J. A. M. A.:* **95,** 707, 1930.
(2) *Am. Jour. Psychiat:* **8,** 783, 1929.
(3) *Am. J. Dis. Child.:* **38,** 451, 1929.

(4) *Bull. No. 175. United States Department of Agriculture*, Office of Experiment Stations: pp. 224, 1907.
(5) *New England Journal of Medicine:* **203,** 145, 1930.
(6) *J. Biol. Chem.:* **57,** 633, 1923.
(7) *J. Am. Chem. Soc.:* **50,** 1625, 1928.
(8) *J. Biol. Chem.:* **63,** 316, 1925.
(9) *Ibidem:* **66,** 375, 1925.
(10) *Ibidem:* **47,** 59, 1921.
(11) *Bull. Johns Hopkins Hosp.:* **XLI,** 389, 1927; *Arch. Neurol. Psychiat.:* **23,** 915, 1930.
(12) *J. Clin. Investigation:* **1,** 359, 1925.
(13) *Tr. Am. Pediat. Soc.:* 1930.
(14) Treatment of Diabetes Mellitus: pp. 272, 1928.

# BIOLOGICAL STUDIES OF THE ACID-FAST ORGANISM[1]

S. A. PETROFF AND WM. STEENKEN, JR.

*From the Research Laboratory, Trudeau Sanatorium, Trudeau, N. Y.*

During the last ten years considerable attention has been given to the instability of bacterial species. It has been pointed out by numerous investigators that the environment in which the organism is cultivated not only plays a very important part in the development of certain cultural characteristics but also has influence on the morphology of the variants. The virulence under certain conditions can be lost and again regained.

The application of dissociation phenomena has enriched our knowledge in clearing up a number of obscure problems which could not be explained in the past. Applying this method of study to the acid-fast organism, we have found that observations made on other bacteria can be repeated also in this group. Non-pathogenic and pathogenic acid-fast organisms have been studied and variants have been isolated and studied.

In his paper we are discussing the dissociation of mycobacterium phlei, mycobacterium tuberculosis avium, mycobacterium tuberculosis bovium, mycobacterium tuberculosis hominis and also an organism which has been extensively used for prophylactic immunization in Europe, known as BCG. The studies up to the present lead us to believe that the acid-fast organisms can be dissociated and at times are unstable. With certainty we can state that environment plays an important part in the development of variants.

From the mycobacterium tuberculosis avium, we have been able to isolate three types of colonies; (1) the "S" which is highly pathogenic for chickens, (2) an intermediate which is only slightly so and (3) the "R" which produces only mild lesions in chickens. We have dissociated the same variants from mycobacterium tuberculosis bovium

[1]Abstract of paper read before The Johns Hopkins Medical Society, December 14, 1930.

and BCG. The various types can be differentiated not only by the topography of the colonies but also by the number of physical properties characteristic of certain types.

One of our associates, Mr. Cooper, recently published the chemical analysis of "R" and "S" colonies, isolated from the Bacillus Calmette-Guérin. His studies clearly demonstrated that the chemistry of these two types of colonies is entirely different.

The various types of colonies are also susceptible to carbon dioxide, oxygen and vapor pressure. When an organism belonging to the acid-fast group is cultivated in a closed circuit, the topography of the colonies differs considerably from that cultivated in open circuit.

The close relationship between various types of acid-fast organisms has attracted our attention, and we believe today that we have no right to state that a certain organism is human, bovine or avian. Furthermore, we believe that the present method for differentiation of the human and bovine is inadequate and that newer methods must be devised for such a differentiation. Our studies so far indicate that the factor of instability must be taken into consideration when discussing any problem in tuberculosis.

# INDEX TO VOLUME XLVIII


Adrenal gland, Congenital syphilis of the ..... 1
Angina agranulocytica and its treatment ..... 339, 349
Atrophy, acute yellow, Diet in preventing, during arsphenamine treatment ..... 131

Bacteria, acid-fast. Their relation to disease and the need for more knowledge ..... 143
Baker, Benjamin M. Jr., and Edward P. Carter, Certain aspects of syphilitic cardiac disease ..... 315
Blackman, Sam S., J. Howard Brown and Geoffrey Rake, The production of acute nephritis by means of a pneumococcal autolysate ..... 74
Book Reviews and Books Received ..... 48, 105, 181, 254
Brenn, Lena, and J. Howard Brown, A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections ..... 69
Bridge, Edward M., and L. V. Iob, The mechanism of the ketogenic diet in epilepsy ... 373
Brown, J. Howard, and Lena Brenn, A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections ..... 69
Brown, J. Howard, Sam S. Blackman and Geoffrey Rake, The production of acute nephritis by means of a pneumococcal autolysate ..... 74

Carter, Edward P., and Benjamin M. Baker, Jr., Certain aspects of syphilitic cardiac disease ..... 315
Cell surfaces, A new stain for ..... 104
Chesney, Alan M., and Thomas B. Turner, Studies in experimental syphilis. IX. The distribution of the resistant state of "immune rabbits." ..... 90
Craven, E. B., Jr., The importance of diet in preventing acute yellow atrophy during arsphenamine treatment ..... 131
Cunningham, R. S., and Edna H. Tompkins, The cells found in tuberculous tissues as demonstrated by vital and supravital staining ..... 8

Dandy, Walter E., Treatment of hemicrania (migraine) by removal of the inferior cervical and first thoracic sympathetic ganglion ..... 357
Di-Hydranol, Control of intestinal putrefaction in man by oral administration of 2-4-dihydroxyphenyl n-heptane ..... 25

Eastman, N. J., and C. M. McLane, II. The lactic acid content of umbilical cord blood under various conditions ..... 261
Egg, A human tubal, unfertilized ..... 368
Epilepsy, The mechanism of the ketogenic diet in ..... 373

Feirer, William A., and Veader Leonard, Di-hydranol. Control of intestinal putrefaction in man by oral administration of 2-4-dihydroxyphenyl n-heptane ..... 25
Firor, Whitmer B., and Charles A. Waters, Roentgenotherapy of angina agranulocytica ..... 349
Fite, George Liddle, Congenital syphilis of the adrenal gland ..... 1
Friedenwald, Jonas S., and Mary L. Small, A new stain for cell surfaces ..... 104

Grafflin, Allan L., The structure of the renal tubule in the toadfish..... 269
Gram-Positive and gram-negative bacteria in tissue sections, A method for the differential staining of..... 69
Guild, Harriet G., The prognosis of acute glomerular nephritis in childhood..... 193

Hamburger, Louis P., Angina agranulocytica and its treatment..... 339
Hemicrania (Migraine) Treatment of, by removal of the inferior cervical and first thoracic sympathetic ganglion..... 357
Hyperthyroidism, A study of the morbid anatomy of hearts from patients dying with..... 273

Iob, L. V., and Edward M. Bridge, The mechanism of the ketogenic diet in epilepsy... 373

Leonard, Veader, and William A. Feirer, Di-hydranol. Control of intestinal putrefaction in man by oral administration of 2-4-dihydroxyphenyl n-heptane..... 25
Lewis, J. K., and Donald McEachern, Persistence of an accelerated rate in the isolated hearts and isolated auricles of thyrotoxic rabbits: response to iodides, thyroxine and epinephrine..... 228
Lewis, Warren H., The outgrowth of endothelium and capillaries in tissue culture..... 242
Lewis, Warren H., A human tubal egg, unfertilized..... 368

Marble, Alexander, Carcinoma of the stomach in a seventeen-year-old girl..... 39
McEachern, Donald, and J. K. Lewis, Persistence of an accelerated rate in the isolated hearts and isolated auricles of thyrotoxic rabbits: response to iodides, thyroxine and epinephrine..... 228
McEachern, Donald, and Geoffrey Rake, A study of the morbid anatomy of hearts from patients dying with hyperthyroidism..... 273
McLane, C. M., and N. J. Eastman, II. The lactic acid content of umbilical cord blood under various conditions..... 261

Nephritis, acute, The production of, by means of a pneumococcal autolysate..... 74
Nephritis, acute glomerular, The prognosis of, in childhood..... 193

Organism, acid-fast, Biological studies of the..... 390
Ovulation in the rabbit, The effect of the injection of urine from pregnant mammals on..... 362

Petroff, S. A., and William Steenken, Jr., Biological studies of the acid-fast organism.. 390

Rake, Geoffrey, Sam S. Blackman and J. Howard Brown, The production of acute nephritis by means of a pneumococcal autolysate..... 74
Rake, Geoffrey, On the pathology and pathogenesis of scleroderma..... 212
Rake, Geoffrey, and Donald McEachern, A study of the morbid anatomy of hearts from patients dying with hyperthyroidism..... 273
Renal tubule, The structure of the, in the toadfish..... 269

Scleroderma, On the pathology and pathogenesis of..... 212
Sino-auricular node and the atrio-ventricular node, On the boundaries of the, in the human heart..... 162

Small, Mary L., and Jonas S. Friedenwald, A new stain for cell surfaces..... 104
Snyder, F. F., and G. B. Wislocki, The effect of the injection of urine from pregnant mammals on ovulation in the rabbit..... 362
Steenken, William Jr., and S. A. Petroff, Biological studies of the acid-fast organism... 390
Stomach, Carcinoma of the, in a seventeen-year-old girl..... 39
Sympathetic nervous system, A search for trophic influence of the, on the adult mammalian skeletal muscle fiber..... 115
Syphilis, experimental, Studies in, IX. The distribution of the resistant state in "immune rabbits"..... 90
Syphilitic cardiac disease, Certain aspects of..... 315

Taussig, Helen B., On the boundaries of the sino-auricular node and the atrioventricular node in the human heart..... 162
Thyrotoxic rabbits, Persistence of an accelerated rate in the isolated hearts and isolated auricles of: response to iodides, thyroxine and epinephrine..... 228
Tissue culture, The outgrowth of endothelium and capillaries in..... 242
Tompkins, Edna H., and R. S. Cunningham, The cells found in tuberculous tissues as demonstrated by vital and supravital staining..... 8
Tower, S. S., A search for trophic influence of the sympathetic nervous system on the adult mammalian skeletal muscle fiber..... 115
Tuberculous tissues, The cells found in, as demonstrated by vital and supravital staining..... 8
Turner, Thomas B., and Alan M. Chesney, Studies in experimental syphilis. IX. The distribution of the resistant state in "immune rabbits"..... 90

Umbilical cord blood, II. The lactic acid content of, under various conditions..... 261

Waters, Charles A., and Whitmer B. Firor, Roentgenotherapy of angina agranulocytica..... 349
White, William Charles, Acid-fast bacteria. Their relation to disease and the need for more knowledge..... 143
Wislocki, G. B., and F. F. Snyder, The effect of the injection of urine from pregnant mammals on ovulation in the rabbit..... 362

# WILLIAM STEWART HALSTED
## SURGEON

By W. G. MacCALLUM

Introduction by Dr. W. H. Welch

*xviii + 242 pages, 15 illustrations. $2.75*

A brief history of the life of the first surgeon of The Johns Hopkins Hospital. Dr. Halsted, who died in 1922, was one of the four men whose names are best known in the establishment of the fame of The Johns Hopkins Medical School and Hospital.

SURGICAL PAPERS OF WILLIAM STEWART HALSTED.

In two volumes, 1232 pages, numerous illustrations. Octavo. $15.00.

When one considers the number, originality and importance of Dr. Halsted's contributions to surgery during the last forty years, which have placed him in the front rank of modern surgeons, and their scattered and often not readily accessible form in various journals and reports, it will be realized that the publication of his collected papers not only will constitute a worthy tribute to his memory, but also will render a welcome and much needed service in making available these significant contributions.

PAPERS AND ADDRESSES. By William Henry Welch.

In three volumes, 2013 pages. Octavo. $20.00.

Dr. Welch's writings are scattered through a great variety of publications, many of which are more or less inaccessible. It was accordingly decided to publish his numerous papers and addresses. These have been collated under the following headings: Vol. I: Pathology, Preventive Medicine. Vol. II: Bacteriology. Vol. III: Medical Education, History, Miscellaneous, Bibliography, Index.

SIR WILLIAM OSLER, BART.

167 pages. Octavo. $1.50.

Brief tribute to his Personality, Influence and Public Service. Written by his friends, associates and former pupils.

HENRY MILLS HURD, THE FIRST SUPERINTENDENT OF THE JOHNS HOPKINS HOSPITAL.

147 pages. Crown octavo. $1.50.

Dr. Thomas S. Cullen has written a short account of Dr. Hurd's medical activities, and yielding to the insistent requests of his friends, Dr. Hurd has added "Random Recollections" of his early life. This volume contains pictures of Dr. Hurd from six to seventy; also an excellent record of the early history of the Johns Hopkins Hospital.

**THE JOHNS HOPKINS PRESS**
**Baltimore, Maryland, U. S. A.**